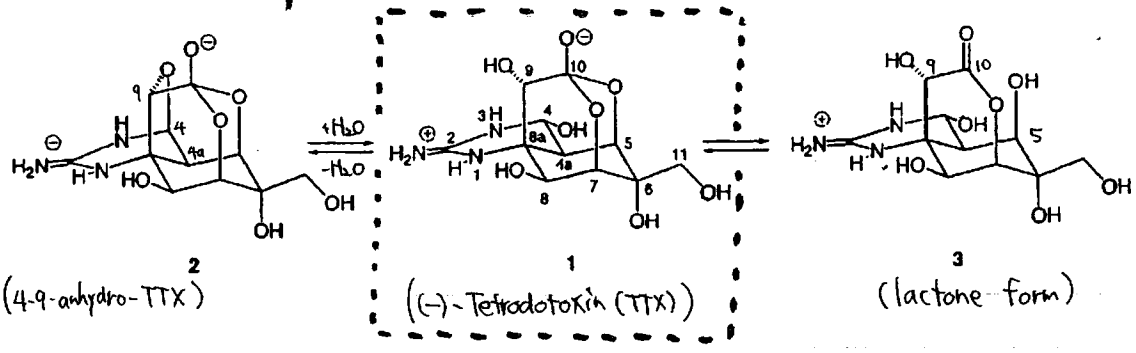
〈Contents〉 ① Introduction
② Comparison of retrosynthesis
③ Isobe's synthesis
(* biological topics)

Wataru Itano (B4)

Literature Seminar

Total Synthesis of Tetrodotoxin (TTX)



2 (4-9-anhydro-TTX)　1 (−)-Tetrodotoxin (TTX)　3 (lactone form)

@ TTX is a toxic principle of puffer fish poisoning (河豚)

Introduction

〈History of TTX〉

Isolation : from the ovaries (卵巣) of the puffer fish Tahara (1909)

Naming : after the puffer fish family "Tetraodontidae" Tahara (1909)
四つ 歯

Structure determination : Hirata-Goto (Kishi) / Tsuda / Woodward (1964) reported at the same time in 国際天然物化学会議

Absolute Stereochemistry : X-ray crystallographic analysis Nitta (1970)

Total syntheses : Kishi-Goto (1972) racemic (J.A.C.S. 1972, 94, 9217-19, 9219-21.)
@ Isobe (2003.1) (J.A.C.S. 2003, 125, 8798-8805)
Du Bois (2003.6) (J.A.C.S. 2003, 125, 11510-11511)

Today's main theme

〈Structual features & properties〉 (adamantane skelton)

* dioxa-adamantane skelton — highly hydroxylated functionarised C×10 … O×5 N×3

* ortho ester (pka=8.7) acidity → zwitter ion
* cyclic guanidine with hemiaminal (about C4)

* equibrium mixture

2 anhydride form ⇌ 1 ortho ester form ⇌ 3 lactone form

〈biological activities〉

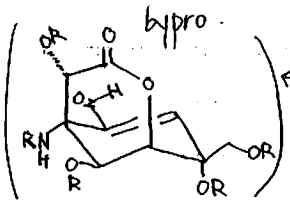
a specific blockage of sodium ion influx through sodium channel protein

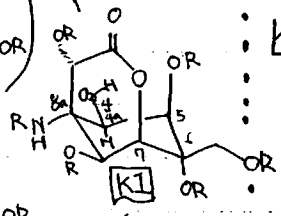
(* hemiaminal base lability
* ortho ester base / acid } lability

Comparison of retrosynthesis

〈Kishi (1972) racemic〉

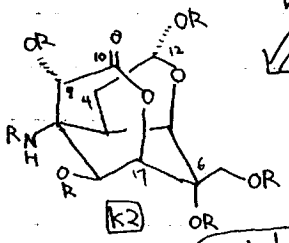
bypro.





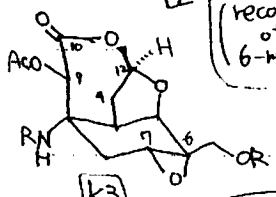
K1

(tetrodamine)



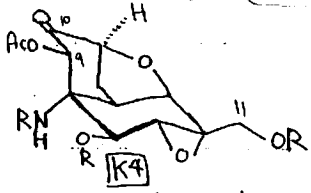
K2

hydrolysis (reconstruction of 6-membered ring)



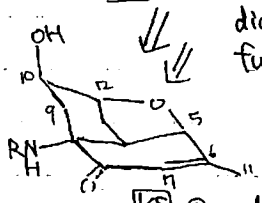
K3

Baeyer-Villiger oxi.



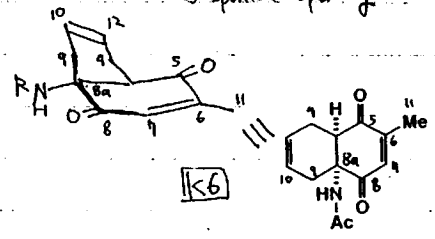
K4

diastereoselective functionalization



K5

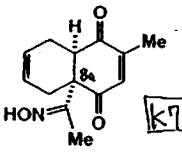
① reduction
② epoxidation
③ epoxide opening



K6

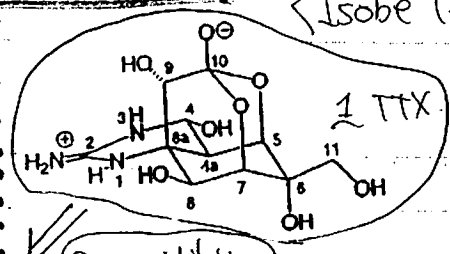
Beckmann reaction

8a N



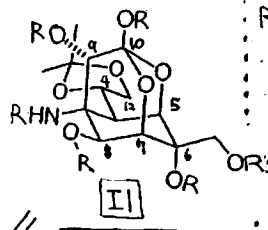
K7

〈Isobe (2003)〉



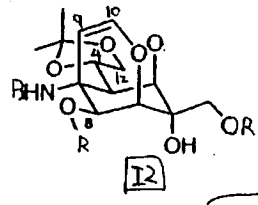
1 TTX

① guanidilation
② formation of hemiaminal



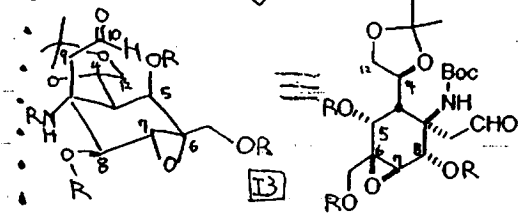
I1

oxi. stereoselective red.



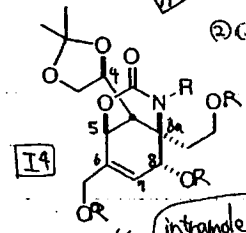
I2

Z-enol attack



I3

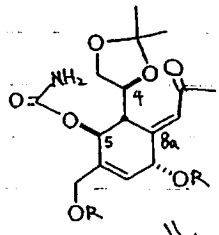
① carbamate cleavage
② C5-OH inversion



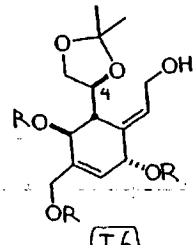
I4

intramolecular conjugated addition

8a N

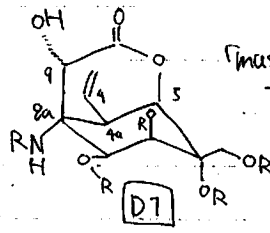


I5



I6

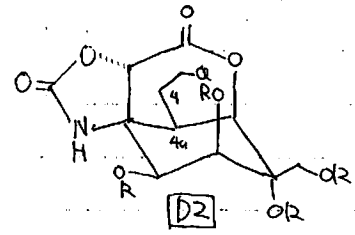
〈Du Bois (2003)〉



D1

masked tetrodamine

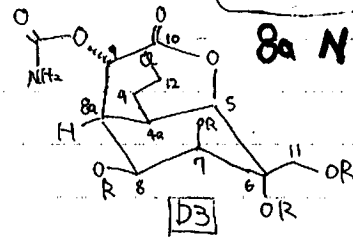
carbamate cleavage
olefin formation



D2

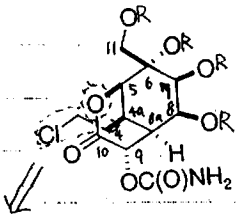
stereoselective Rh-nitrene C-H insertion

8a N

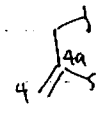


D3

|||



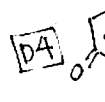
allylic oxi.



Tebbe reagent

C4a-C4 bond

C skelton

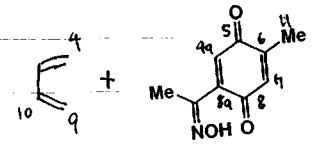


D4

<Kishi>

K7 **C-skelton 6-ring**

⇓ Diels alder reaction (C4–C4a / C9–C8a) bond

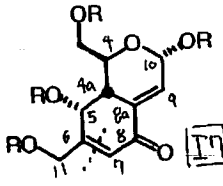


K8
||
SM

<Isobe>

I6

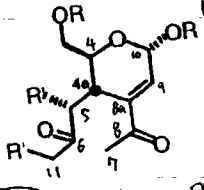
⇓ acetal cleavage reduction



I7

⇓ aldol condensation (C6–C7 bond)

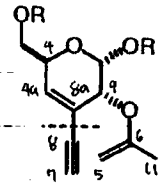
6-ring C skelton



I8 R' = OH
I9 R' = H

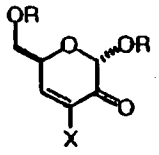
⇐ selective oxidation

⇓ claisen rearrangement (C4a–C5 bond)



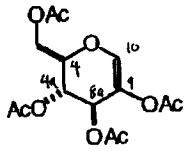
I10

⇓ Sonogashira coupling (C8a–C8 bond)



I11

⇓ (known)

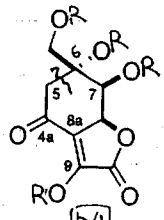


I12
||
SM

<Du Bois>

D3

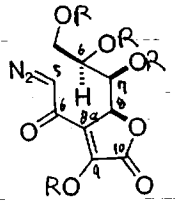
⇓



D4

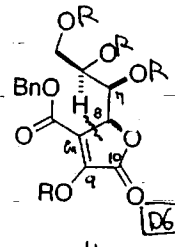
6-ring C5–C6 bond

⇓ Stereoselective Rh-carbene C-H insertion



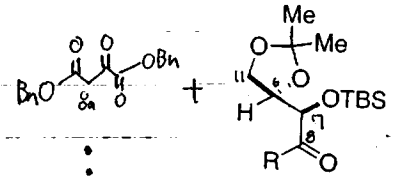
D5

⇓



D6

⇓ C8–C8a bond



D7
||
SM

"First-Asymmetric"

"A stereoserective"

J|A|C|S
ARTICLES
Published on Web 06/28/2003

First Asymmetric Total Synthesis of Tetrodotoxin

Norio Ohyabu, Toshio Nishikawa, and Minoru Isobe*

Contribution from the Laboratory of Organic Chemistry, School of Bioagricultural Sciences, Nagoya University, Furocho, Chikusa, Nagoya 464-8601, Japan

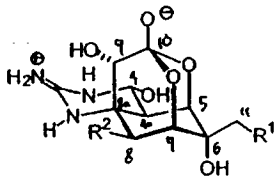
Received January 23, 2003; E-mail: isobem@agr.nagoya-u.ac.jp

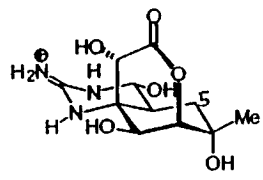
4

10. Previus analogue Synthesis)

11-deoxy TTX (J.A.C.S. 2002, 124, 7847)
8-11-dideoxy TTX (O.L. 2002, 4, 2679.)
5-11-dideoxy TTX (Angew. Chem. Int. Ed, 1999, 38, 3081.)
(Tetrahedron. 2001, 57, 4543.)

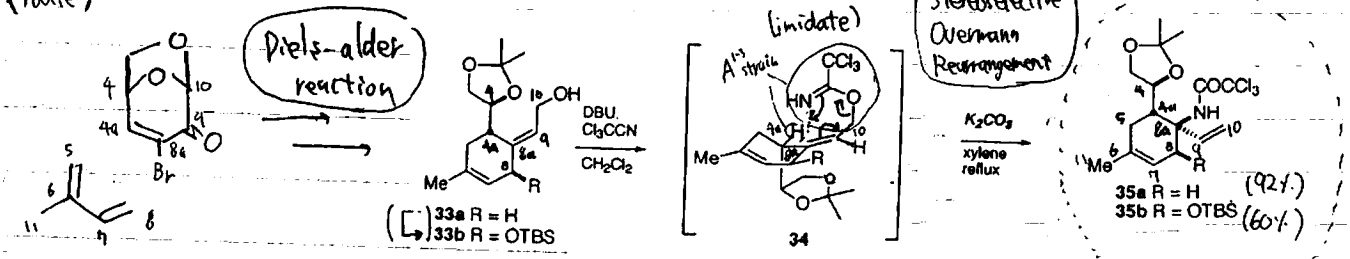
TTX analogues





(−)tetrodotoxin (TTX, 1) R^2 = OH R^1 = OH
11-deoxyTTX R^2 = OH R^1 = H ← from 35b
8,11-dideoxyTTX R^2 = H R^1 = H ← from 35a
5,11-dideoxyTTX

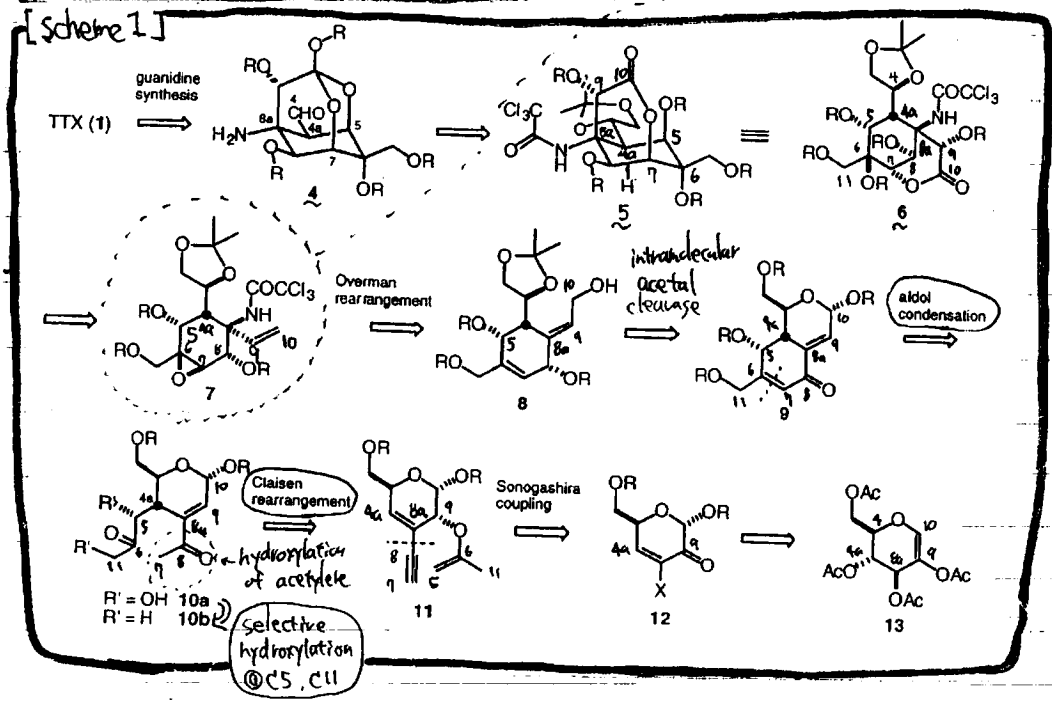
(route)



I. Retrosynthesis and Synthetic Plan

similar

[Scheme 1]



1⇒4 through the chemistry of analogue synthesis

4⇒5 ortho ester formation
① prevent C5-OH from β-elimination
② inhibit epimerization of C9

6⇒7 intramolecular epoxide opening
(C10-O⊖ → C7)

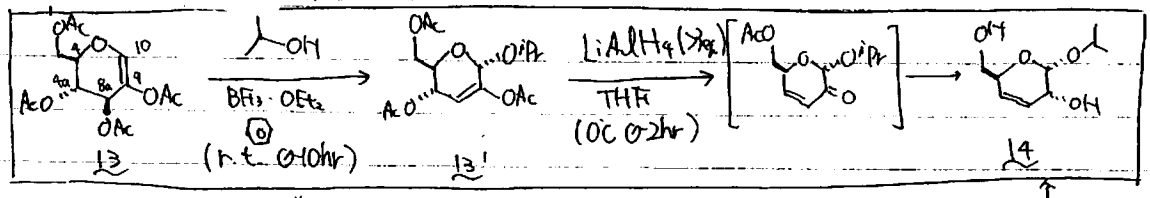
7⇒8
Overmann Rearrangement
(key) (actually problematic)

@ For stereocontrolled synthesis of 8, they invented new route 8⇒13
(aldol condensation, Claisen Rearrangement, hydroxyreation of acetylene
selective hydroxylation, Sonogashira coupling

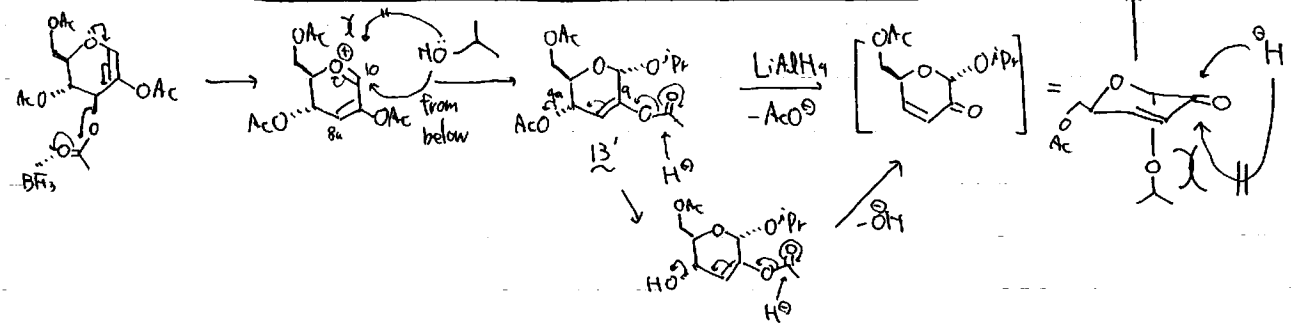
Ⅱ. Synthesis of the Cyclohexane Skelton

(* 13 chiral SM
(-)-2-acetoxy-tri-O-acetyl-D-glucal
1g #20
5g #19960 (Lancaster Synthesis))

* Preparation of 14

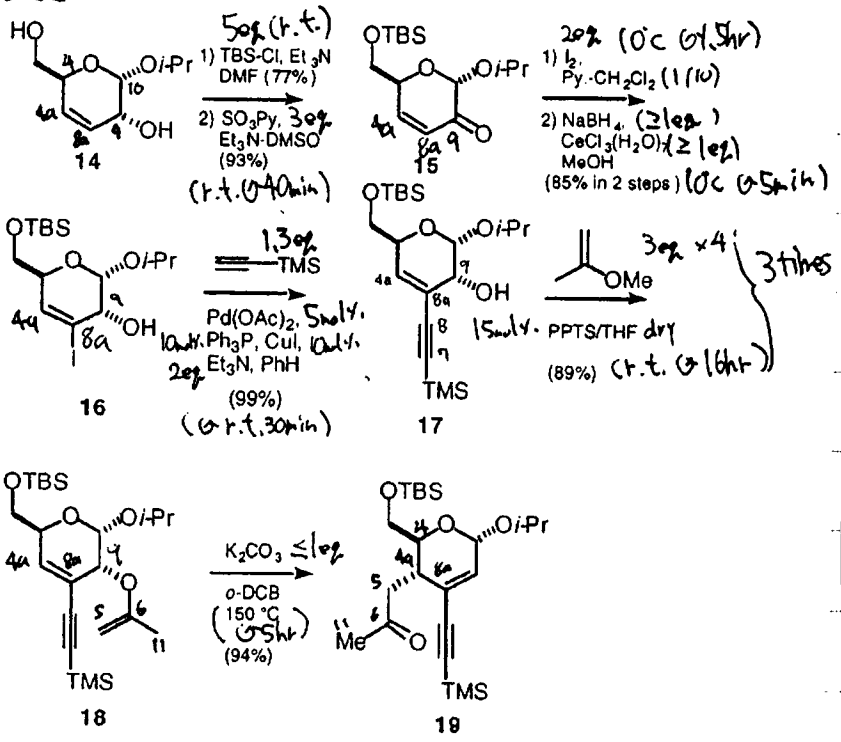


(Tetrahedron 1998, 89, 5505.)



* Sonogashira Coupling and Claisen Rearrangement Strategy

[Scheme 2]



14 → 17 formation of C8a-C8 bond via Sonogashira Coupling

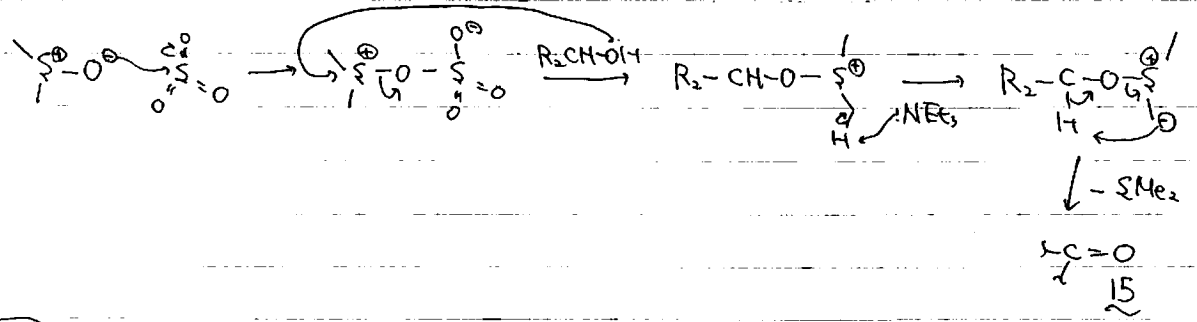
17 → 19 formation of C4a-C5 bond via Claisen Rearrangement

14→15

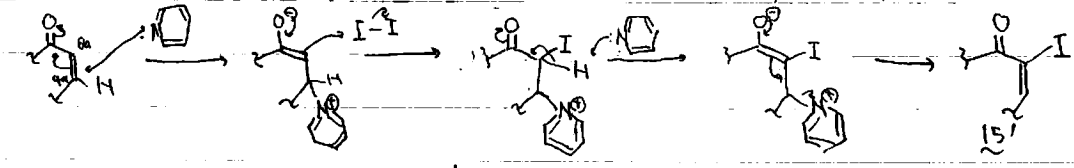
1) 1° alcohol selective protection

2) Parlich-Doeling oxidation (J.A.C.S. 1967, 89, 5505.)

(suitable for γ-OH → =O , γ-OH → >C=O)



15→16 1) α-iodination of enone 15 (T.L. 1987, 43, 4737.)



2) reduction under nucle condition. (J.A.C.S. 1981, 103, 5454.)

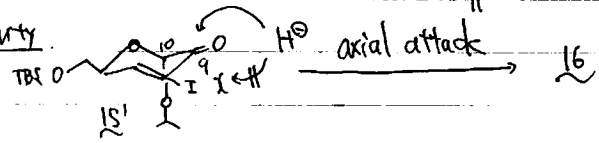
Ce^{3+} ion — accelerate

$NaBH_4 + n\,MeOH \longrightarrow NaBH_{4-n}(OMe)_n + n\,H_2$ · (n = 1, 2, 3)

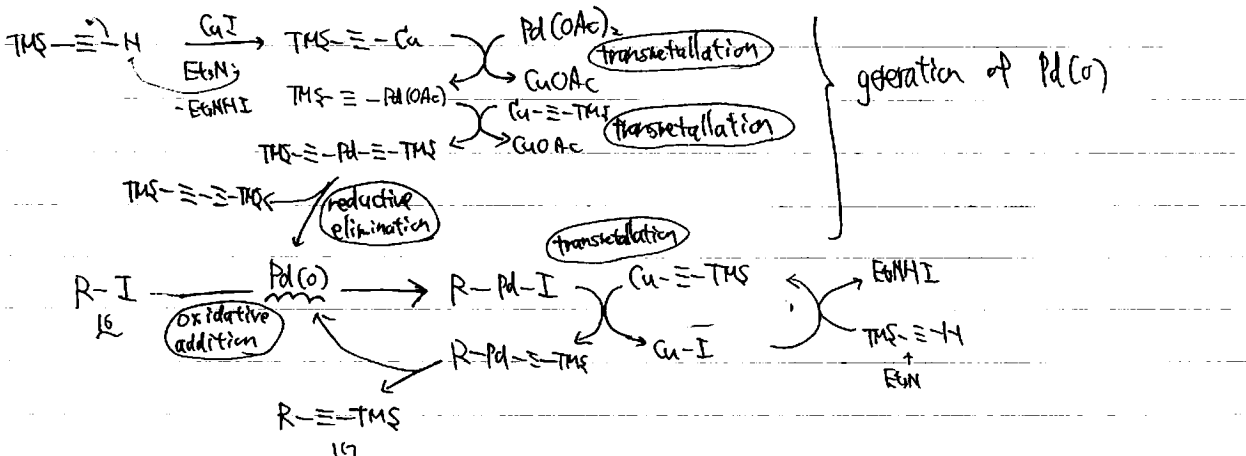
regioselectivity. real reducting reagent

n↑ ⇒ reagent の hard 性↑ ⇒ より hard な site に対して 1,2-attack

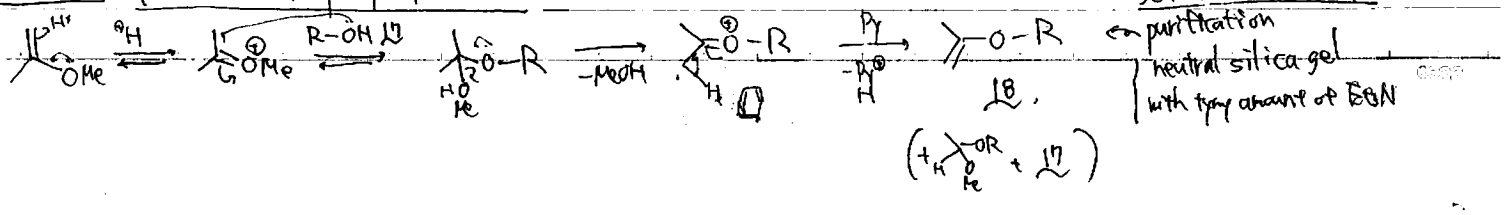
stereoselectivity.



16→17 Sonogashira coupling. (T.L. 1975, 43, 4737.)



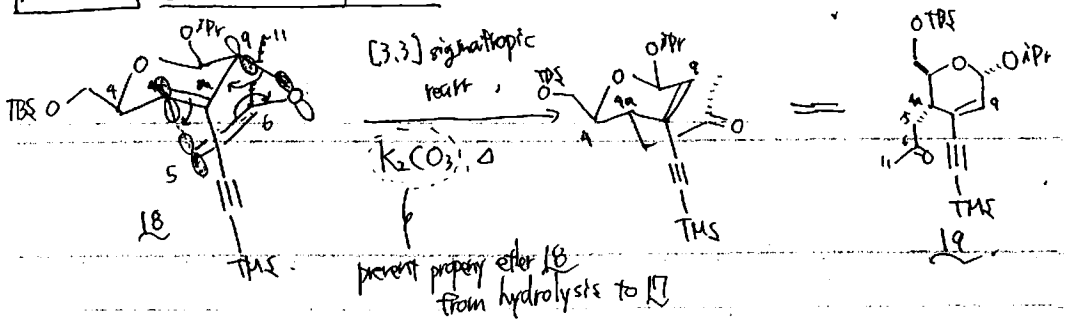
17→18 formation of propargyl ether



sensitive to acid

purification: neutral silica gel with trace amount of EtN

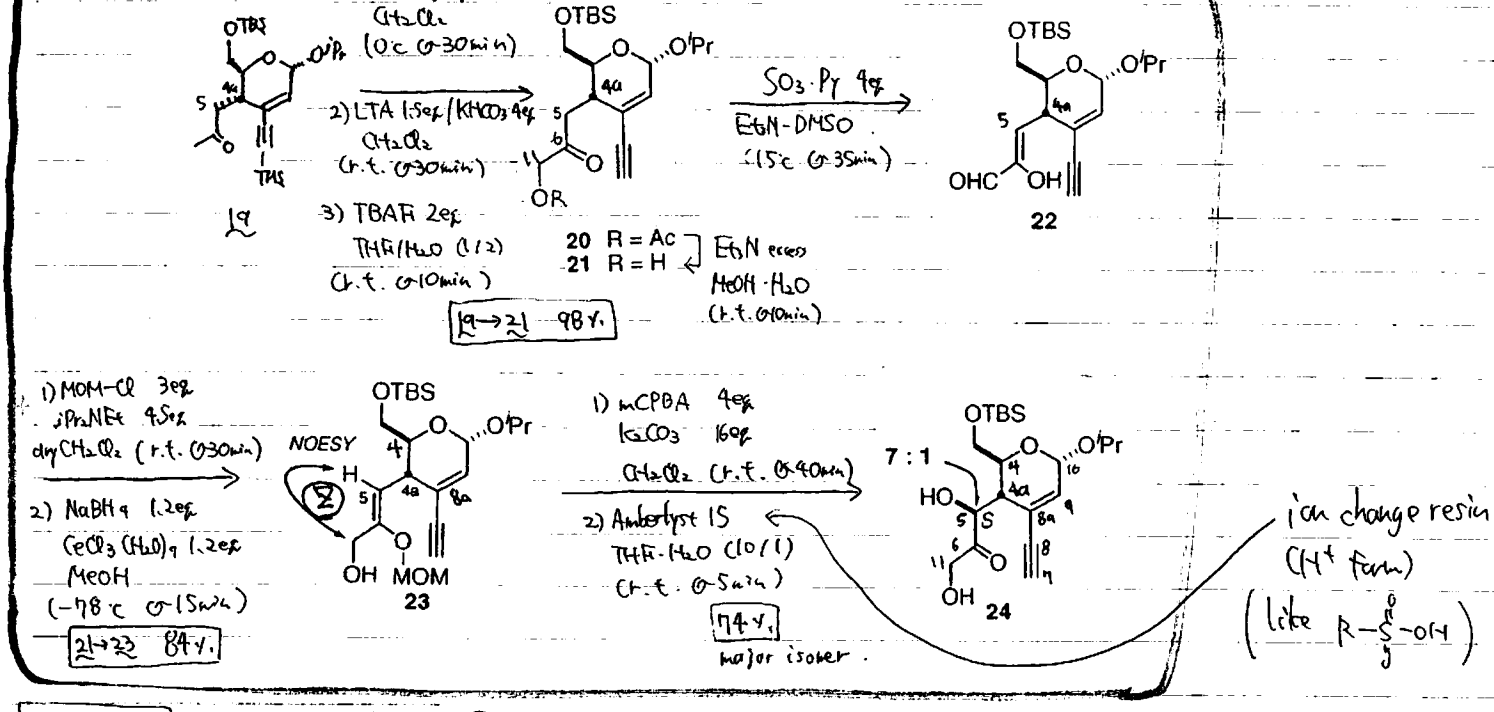
18 → 19 Claisen Rearrangement



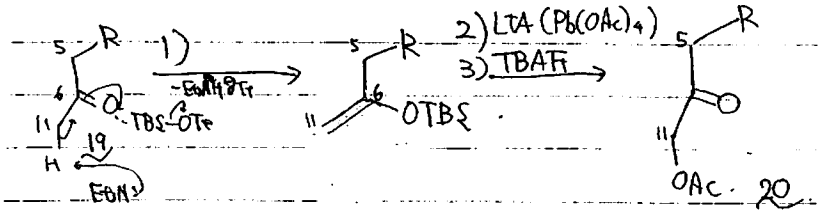
✱ Stereoselective Oxigenation of C5 and C11

poor enoliazable : oxygenation difficult

[Scheme 3]

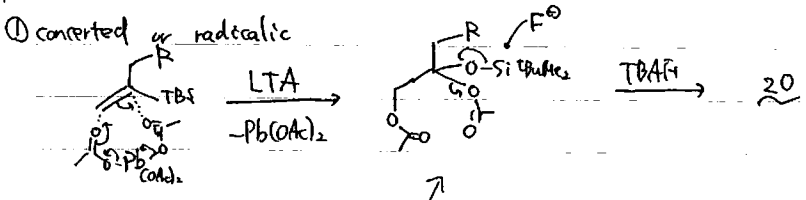


19 → 20 Introduction of OAc @C11

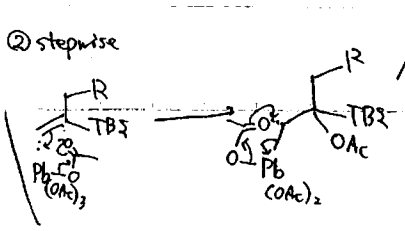


(2), 3) (Synth. Commun. 1976, 6, 59.) (J. O. C. 1976, 41, 1673)

① concerted or radicalic

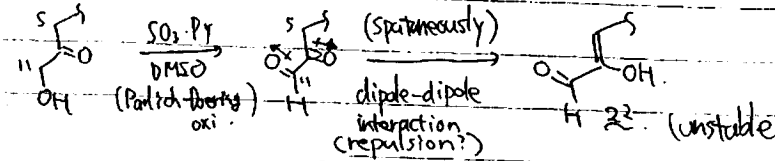


② stepwise

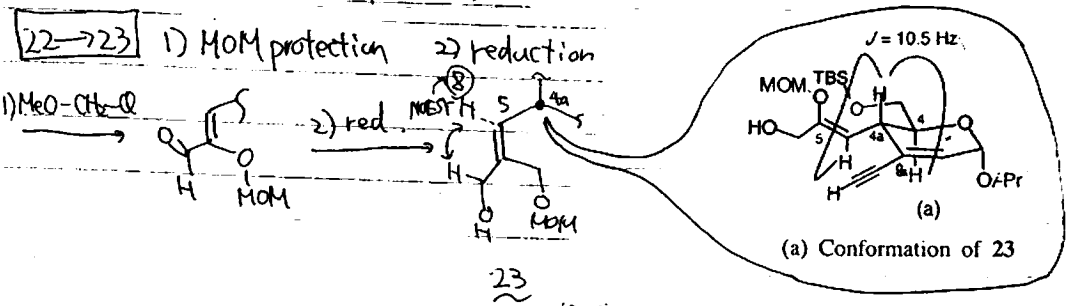


20→21 deacetylation

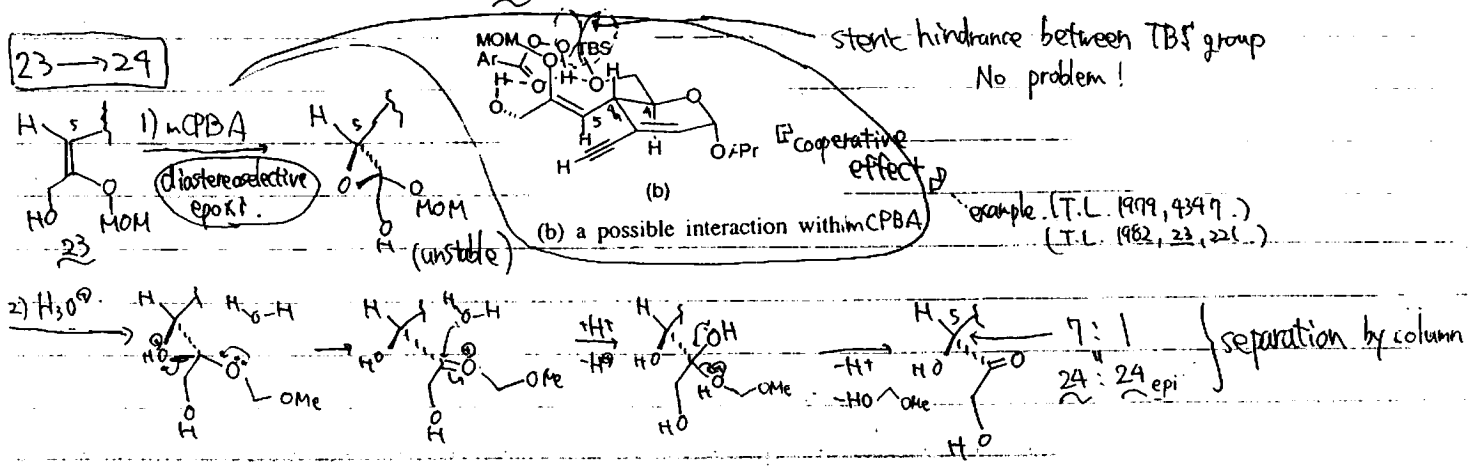
21→22 enolization toward internal C5 ← poor enolizable.



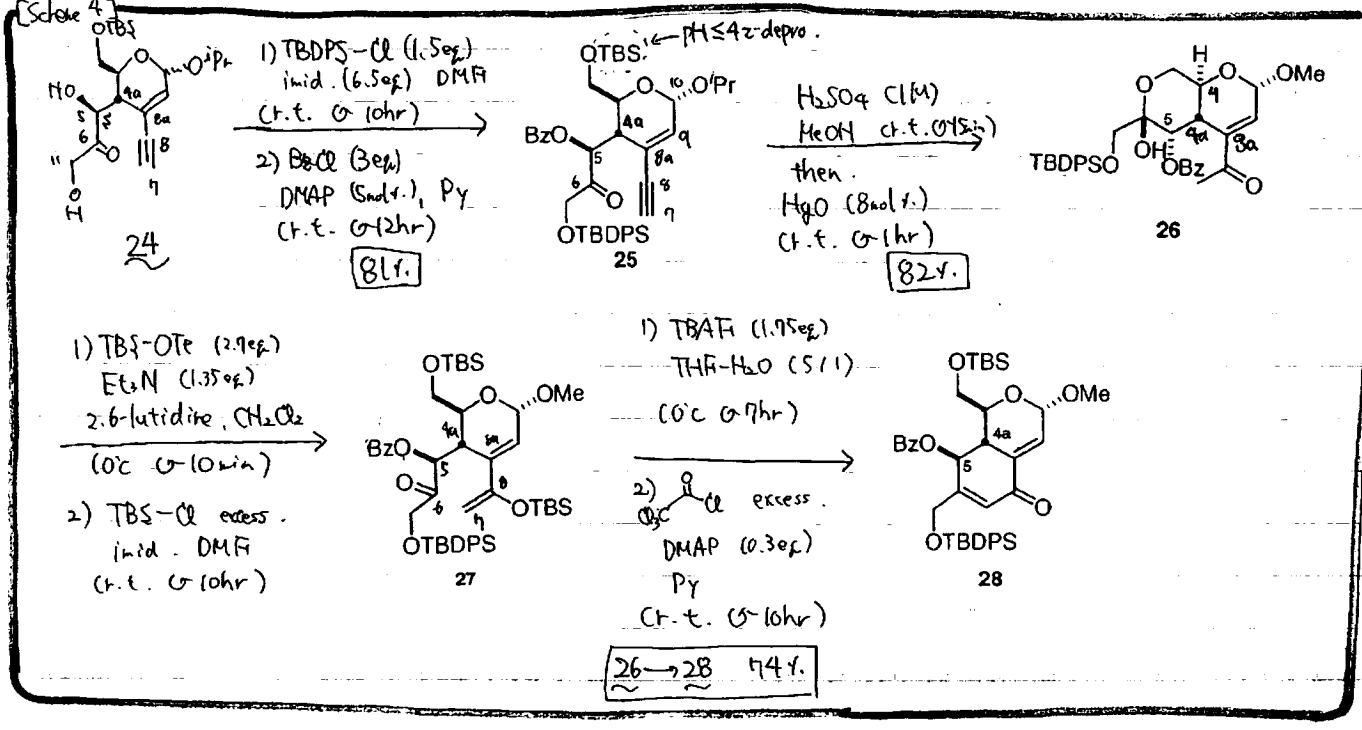
22→23 1) MOM protection 2) reduction



23→24



✱ formation of C6-C7 bond via Claisen condensation

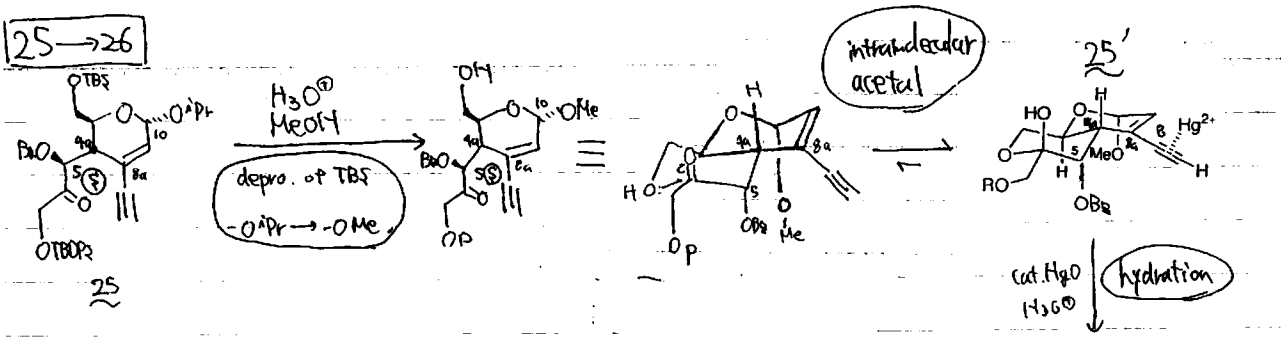


24 → 25

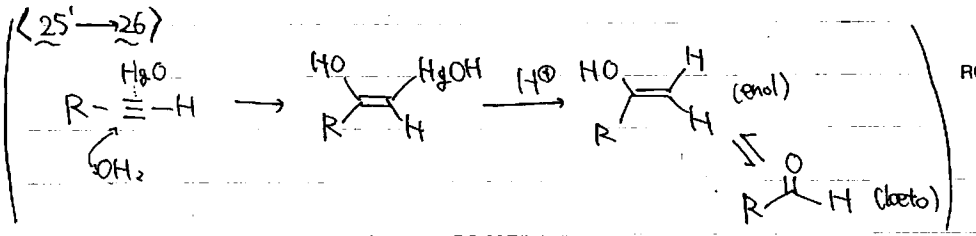
1) Selective protection of 1° alcohol (@C11) by TBDPS group

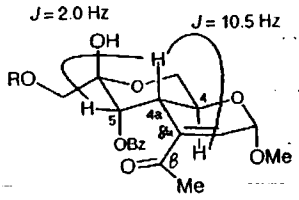
2) Benzoylation of 2° alcohol (@C5)

25 → 26



〈25' → 26〉

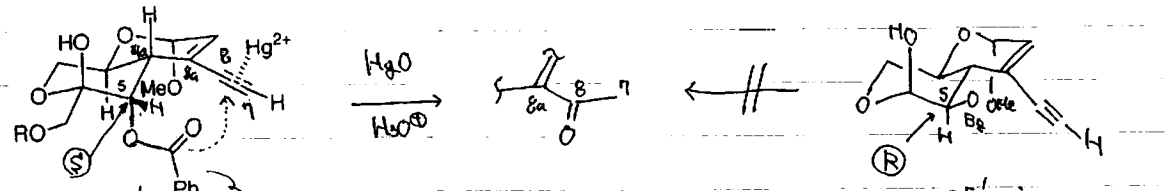




configuration determined ⇓ 24 C5 = (S)

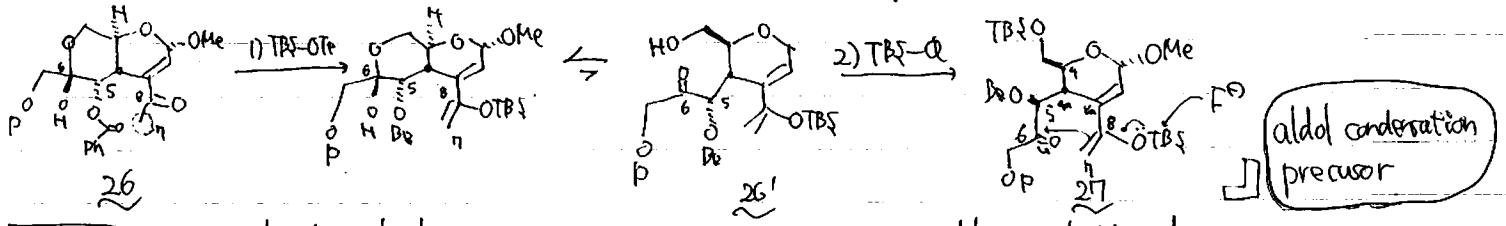
if C5 = R ⇒ this hydration of acetylene didn't occur.

So this interaction might accelerate the reaction.

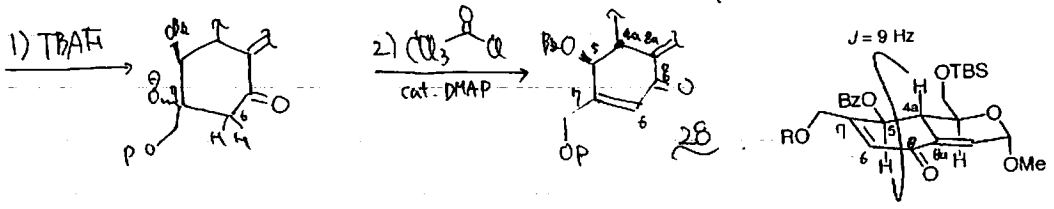


26 → 27

1) alkenyl-silyl ether formation 2) 1°-OH selective protection



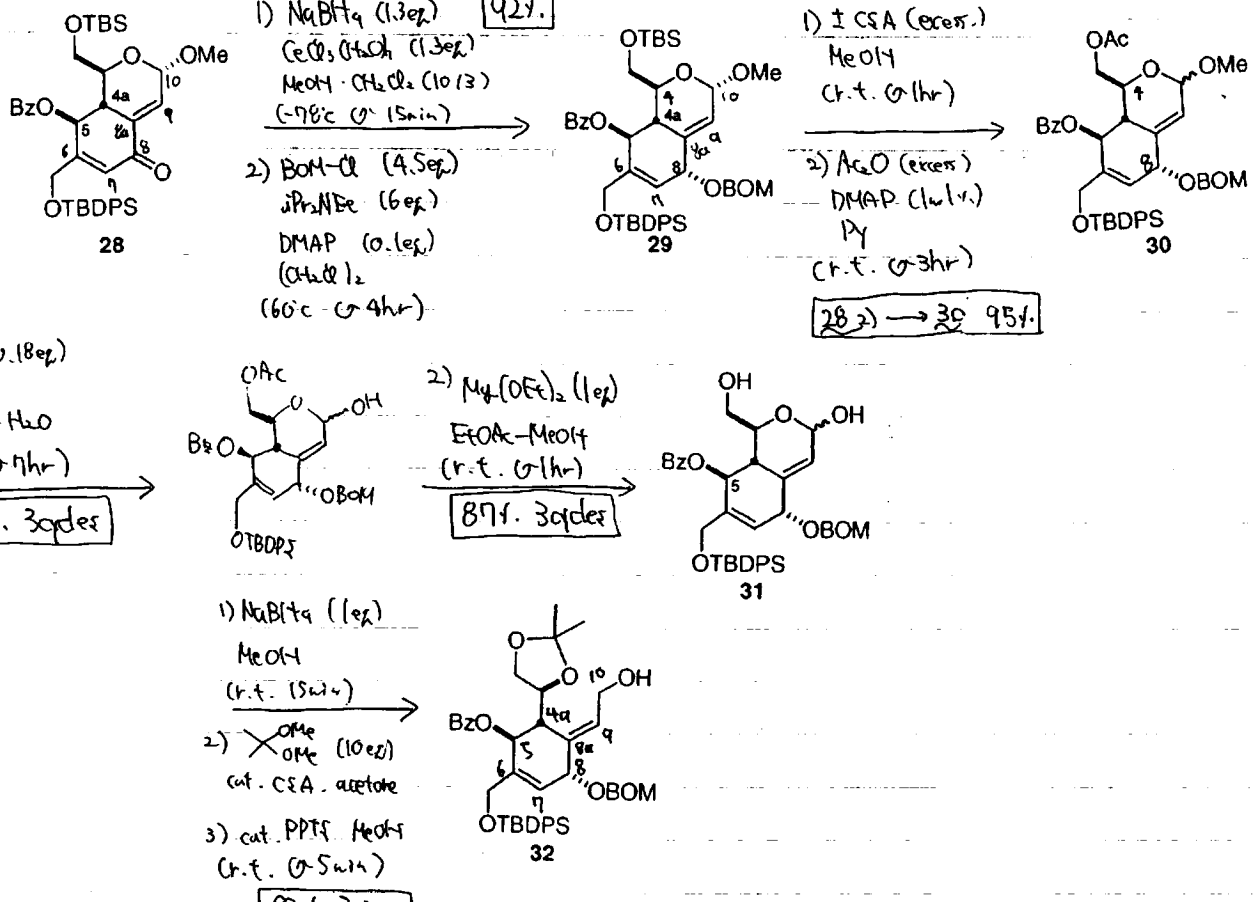
27 → 28 1) Intramolecular aldol reaction 2) dehydration with trichloroacetyl chloride



⇒ C5 stereochemistry determined

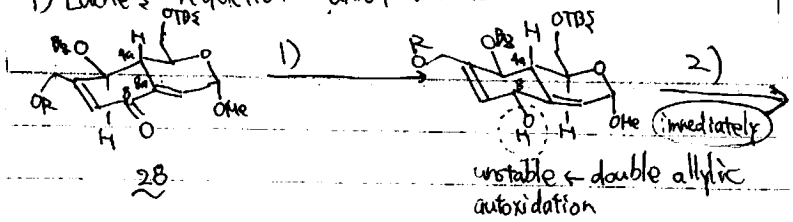
✱ Exooletin Synthesis

[Scheme 5]

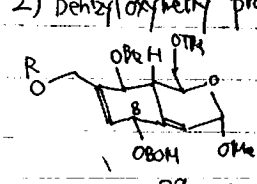


28 → 29

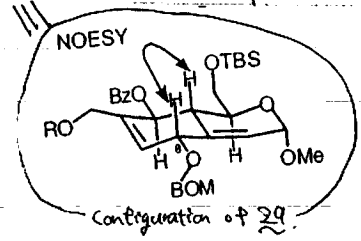
1) Luche's reduction - axial attack.



2) Benzyloxymethyl protection of C-8 alcohol



BOM : ① low leaving ability
② high compatibility
③ mild deprotection condition



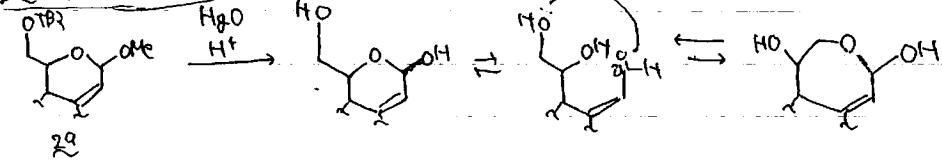
Configuration of 29

29 → 30

1) deprotection of TBDMS, then 2) Acetylation.

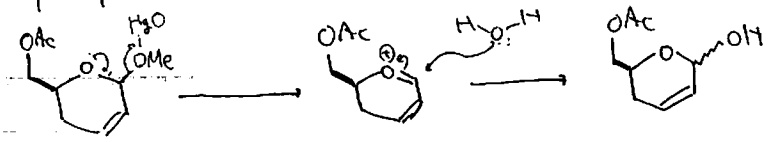
if TBDMS remains.....

under 30 → 31 1) condition



30 → 31

1) Hydrolysis of the acetal ⇒ hemiaminal

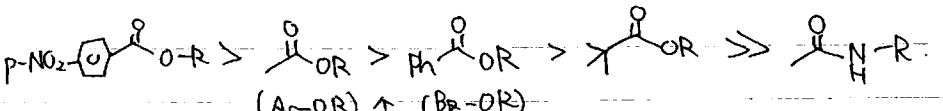


30

* in the absence of HgO
harsh condition necessary
(CSA (100 mg/mL) in H_2O–acetone. r.t. 1 day)

2) mild deacetylation (T.L. 1996, 37, 455).

reactivity of deacylation



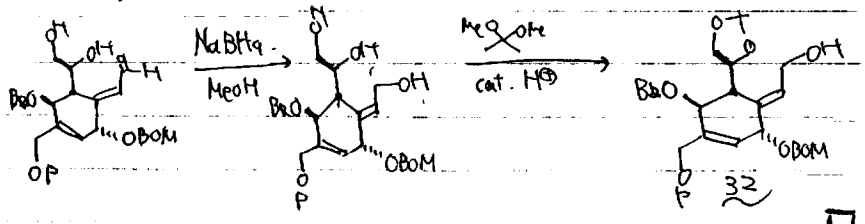
(Ac–OR) ↑ (Bz–OR)

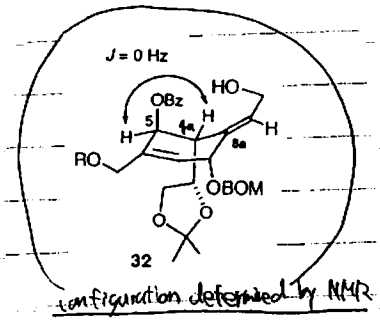
this time

@ equivalent of $Mg(OMe)_2$ → controll selectivity

31 → 32

1) reduction, then 2) acetonide formation





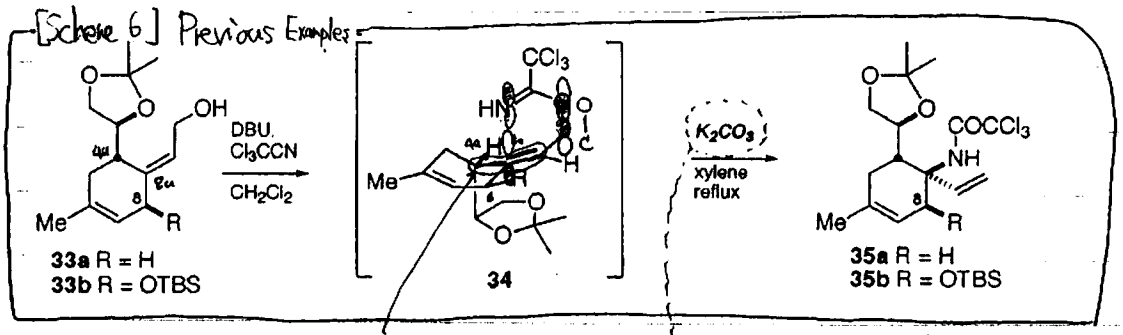
configuration determined by NMR

Formation of Cyclohexane Skeltone for next step Completed.
(Institution of N)

II. Introduction of Nitrogen Functionality

★ Overman Rearrangement Strategy **failed**

[Scheme 6] Previous Examples:



33a R = H
33b R = OTBS

34

35a R = H
35b R = OTBS

$A^{1,3}$ strain ↑↑

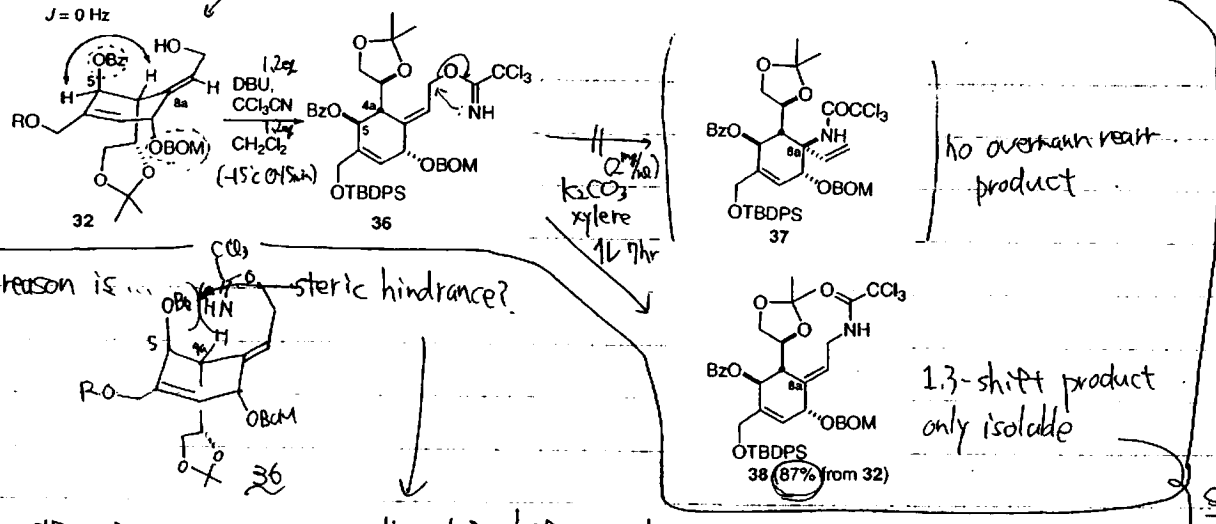
trap acids during thermal rearrangement (J.O.C. 1983, 63, 188.)
(like )

↑
detected (xylene reflux overnight)

But ... (DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene)

This case.

[Scheme 7] — compared with previous one
* axial -OBz @C5
* axial C8-OH → eq. C8-OH



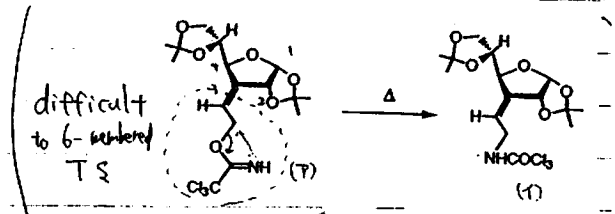
The reason is ... steric hindrance?

But C5 epimer gave corresponding 1,3-shift product.

⇓

(?) Anyway, They gave up this strategy.

similar example:
in the presence of high steric hindrance, 1,3 shift occured.

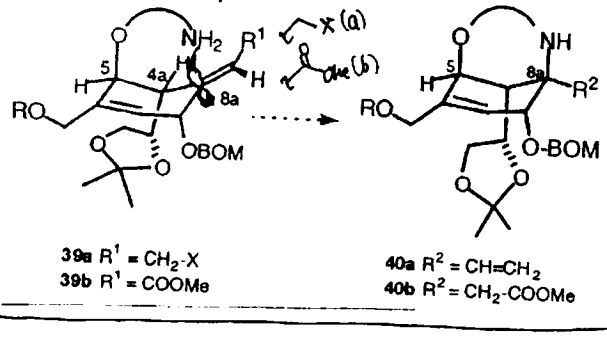


(有機化. 1999, 38, 5569)

* Intramolecular Conjugate Addition Strategy

succeeded

[Scheme 8] Concept



32 ⟶ 41

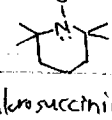
1) ~~removal of benzoate @C5~~

2) 1° alcohol selective oxidation @C10 to aldehyde
(J.O.C. 1996, 61, 7452.)

@under biphasic conditions (CH_2Cl_2–water)
with TBACl as a phase transfer catalyst

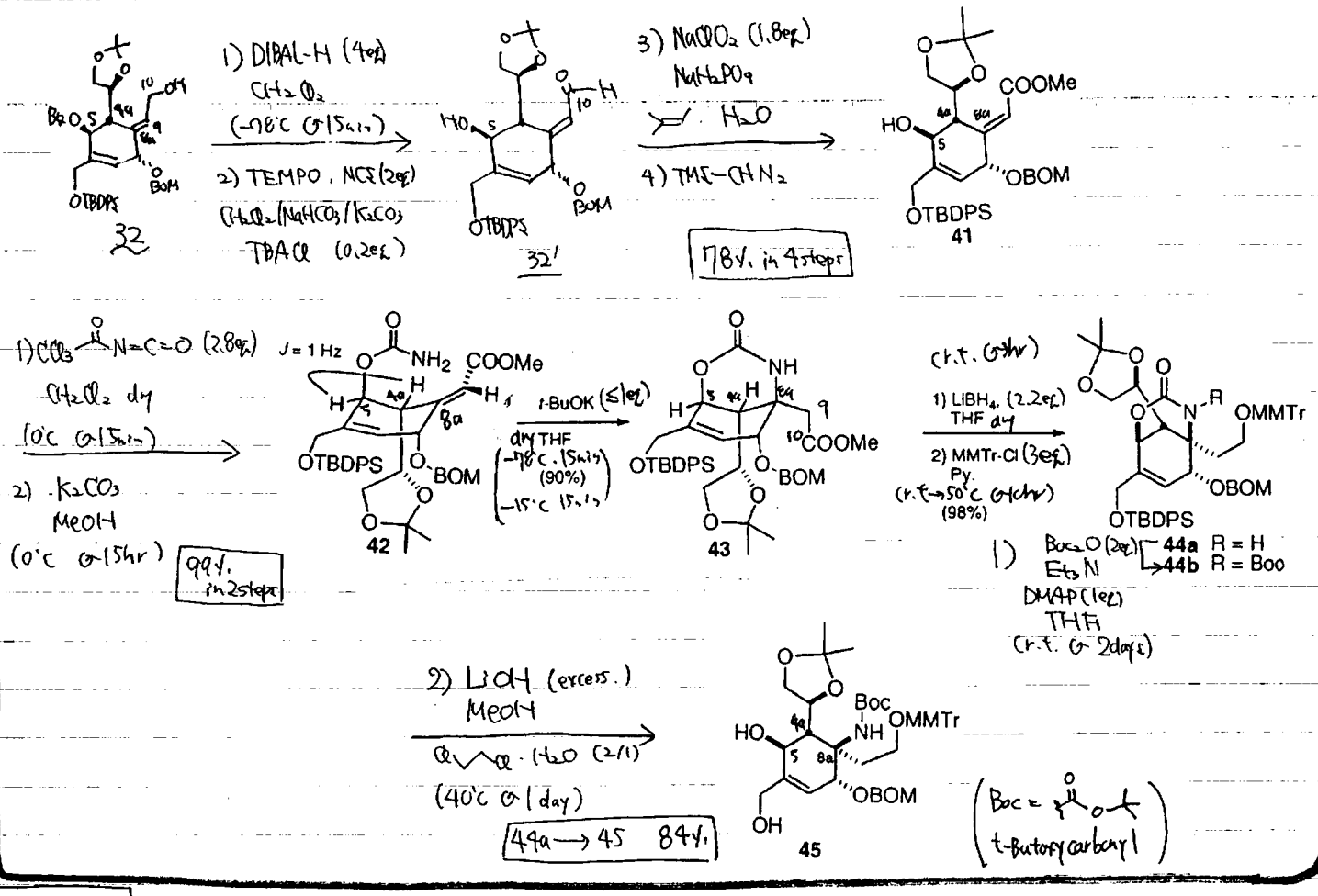
⇒ mechanism ?? (free radical oxidation)

(TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy) .. cat



(NCS = N-chlorosuccinimide) ... stoichiometric oxidant

[Scheme 9]

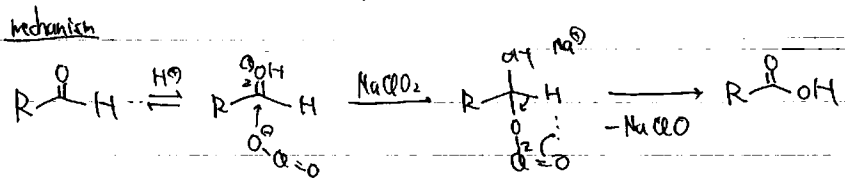


32' → 41

3) Oxydation of aldehydes to carboxylic acid with chlorite (in the presence of -OH)

(Acta. Chem. Scand. 1973, 27, 888.)

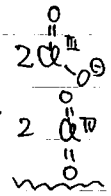
Mechanism



(化)	$HO-Cl(=O)_2$	$HO-Cl(=O)$	$HO-Cl=O$	$HO-Cl$
酸	perchloric acid (過塩素酸)	chloric acid (塩素酸)	chlorous acid (亜塩素酸)	hypochlorous acid (次亜塩素酸)
(塩)	perchlorate	chlorate	chlorite	hypochlorite

(Cl_2) ... chlorine scavenger

if Cl_2 exists ...

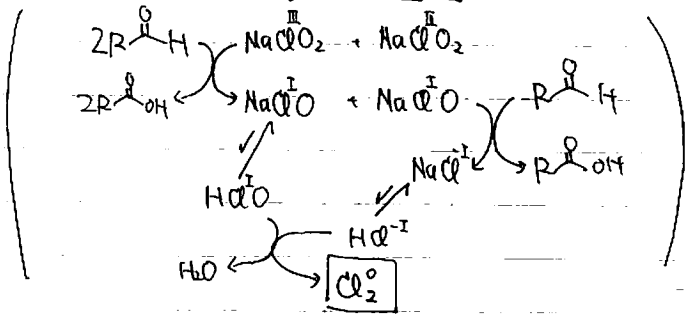


chlorite was oxidised to chlorine dioxide

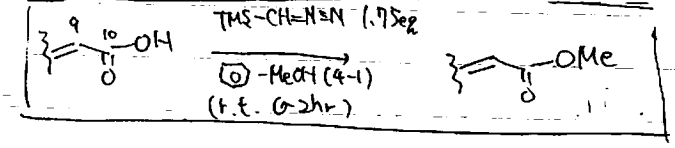
↓ much oxidisable

3) continued ...

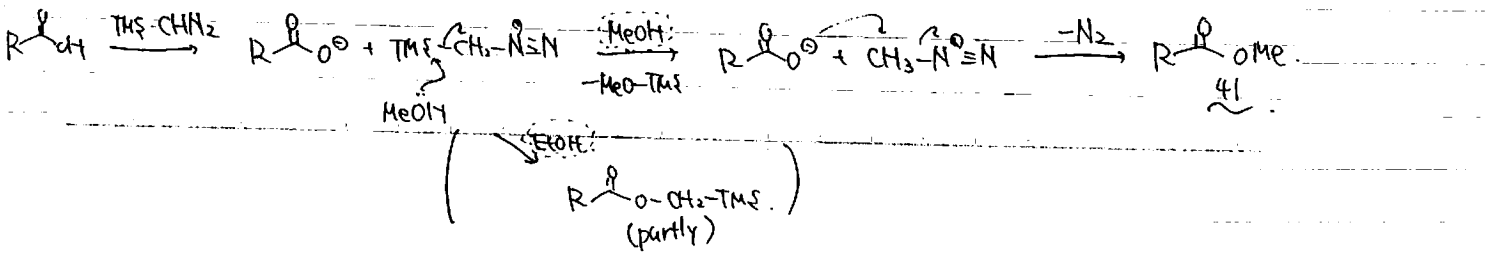
② How chlorine generates?



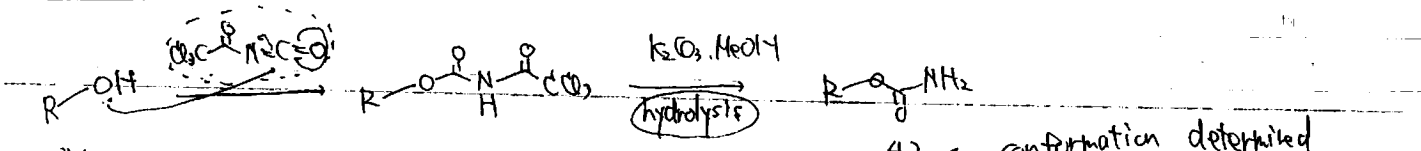
4) Methylation of carboxylic acid



(Chem. Pharm. Bull. 1981, 29, 1495.)



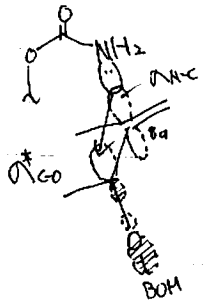
41 ⟶ 42 C5-OH ⟶ carbamate (T.L. 1986, 27, 5521.)



42 ← conformation determined by C4a-C5 coupling constant. H H

42 ⟶ 43

Intramolecular conjugated addition



High yield of the reaction at low temperature.

① Conformation (see scheme 8)

② forming σN-C8a bond ⟷ antiperiplanar orbital interaction ("stereoelectronic effect")

neighboring σ*C-O bond

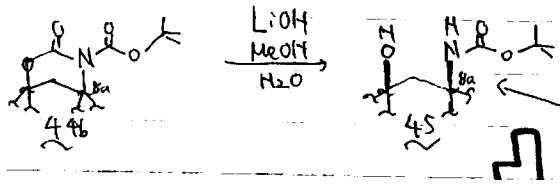
↳ reaction was promoted.

43 ⟶ 44a 1) reduction of ester @ C10

2) MMTr (p-methoxyphenyl)diphenyl methyl (= MeO-C6H4-C(Ph)2-) protection of alcohol

44a ⟶ 45 1) formation of N-Boc

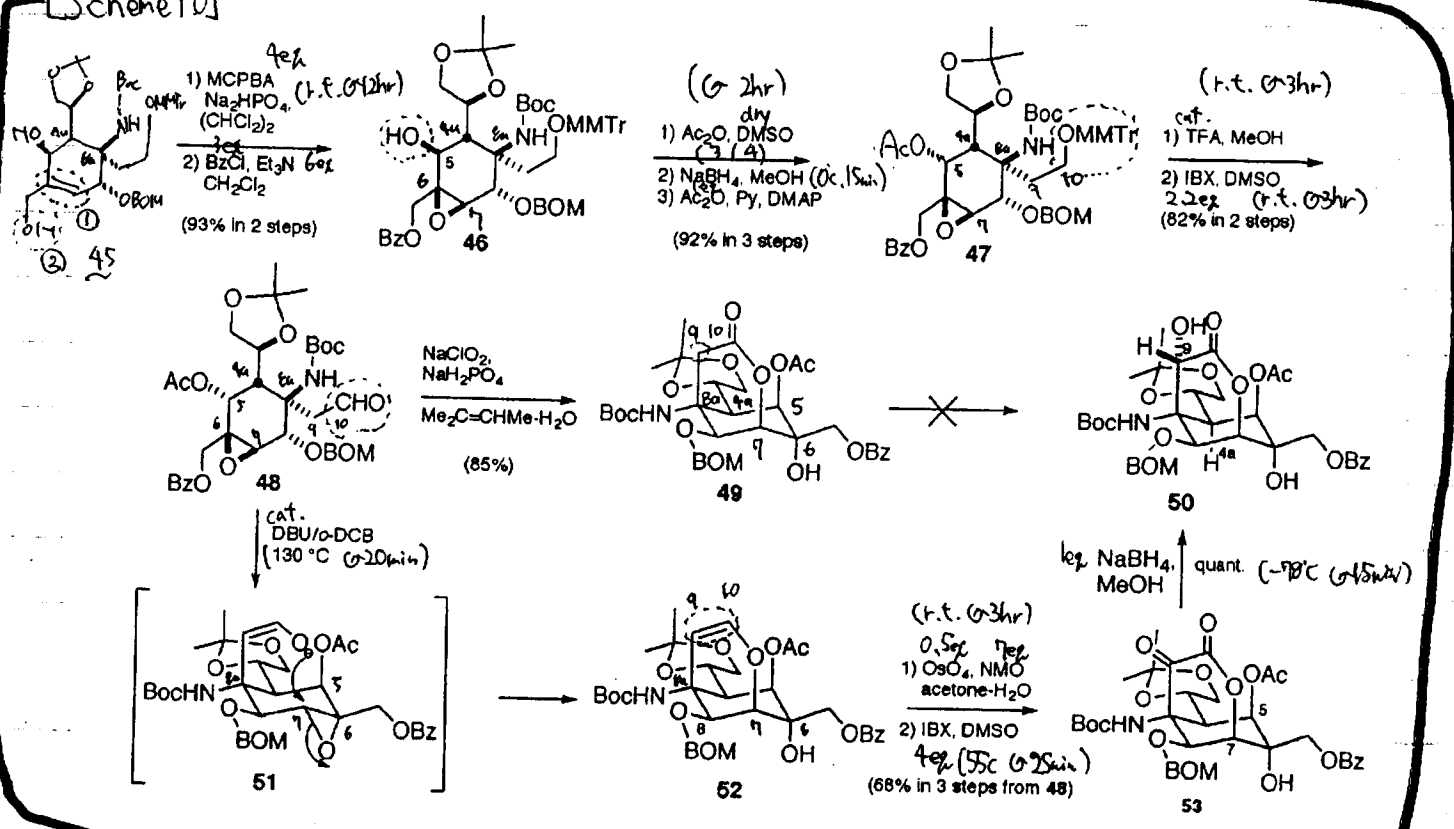
2) hydrolysis of carbamate, TBDPS deprotection.



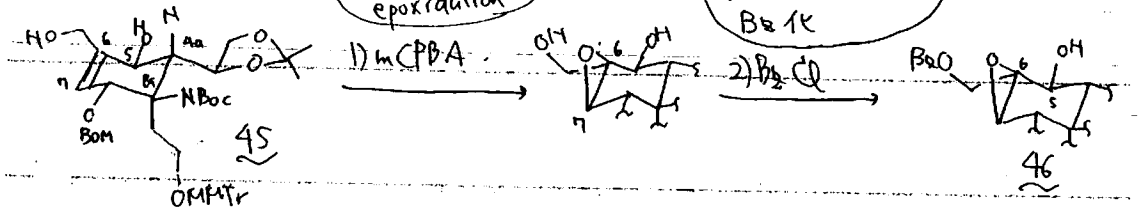
Now they can introduce N-atom @ C8a

IV. Stereoselective Synthesis of the Lactone Ring

[Scheme 10]



45 → 46

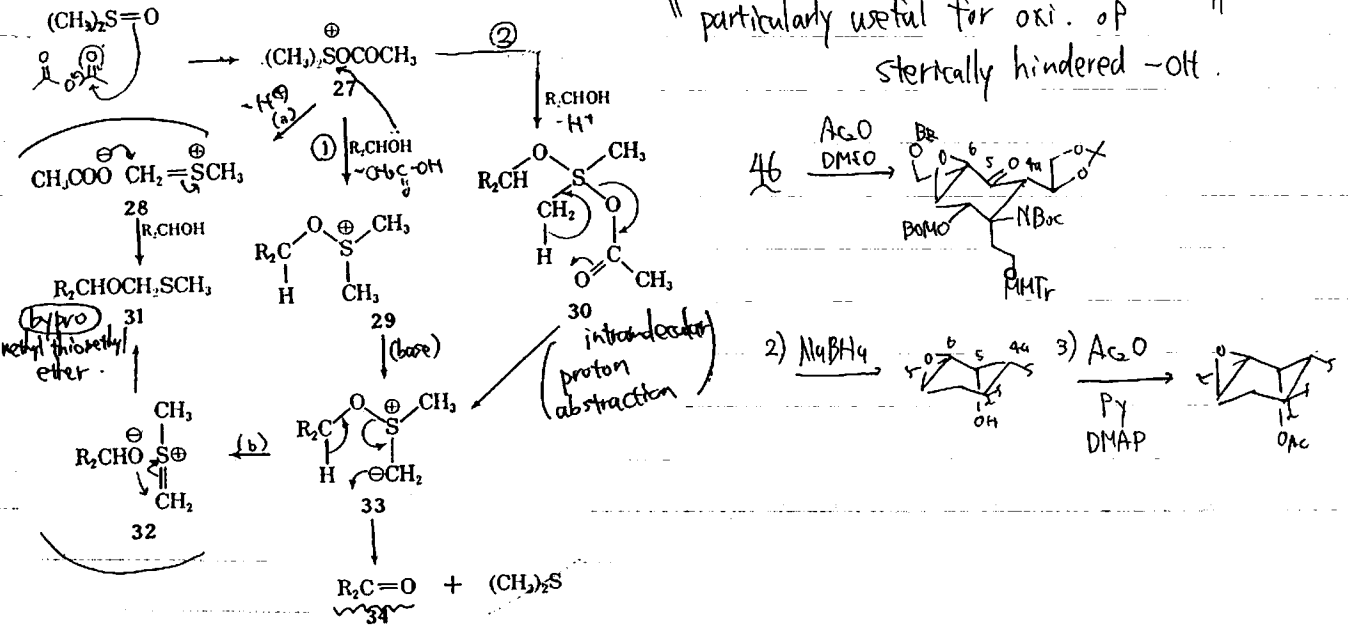


46 → 47

(cf 14 → 15 ≥)

1) Albright–Goldmann oxidation (J.A.C.S. 1965, 87, 4214. / 1967, 89, 1944, 2416.)

" particularly useful for oxi. of sterically hindered -OH. "

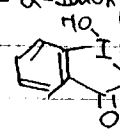


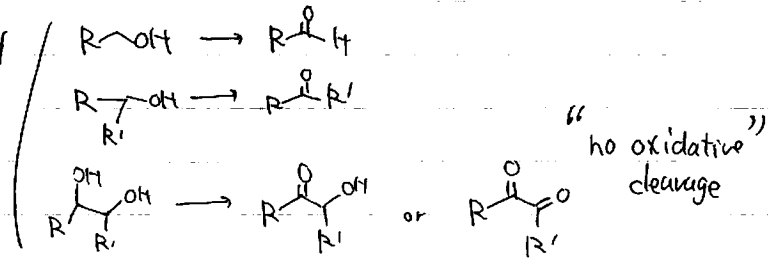
47 → 48

1) acidic deprotection of MMTr ()

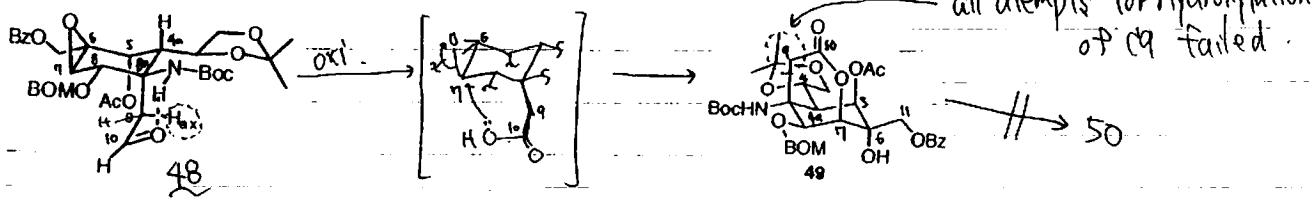
2) oxidation of C10–OH to aldehyde

(J.O.C. 1995, 60, 7272. / T.L. 1994, 35, 8019.)

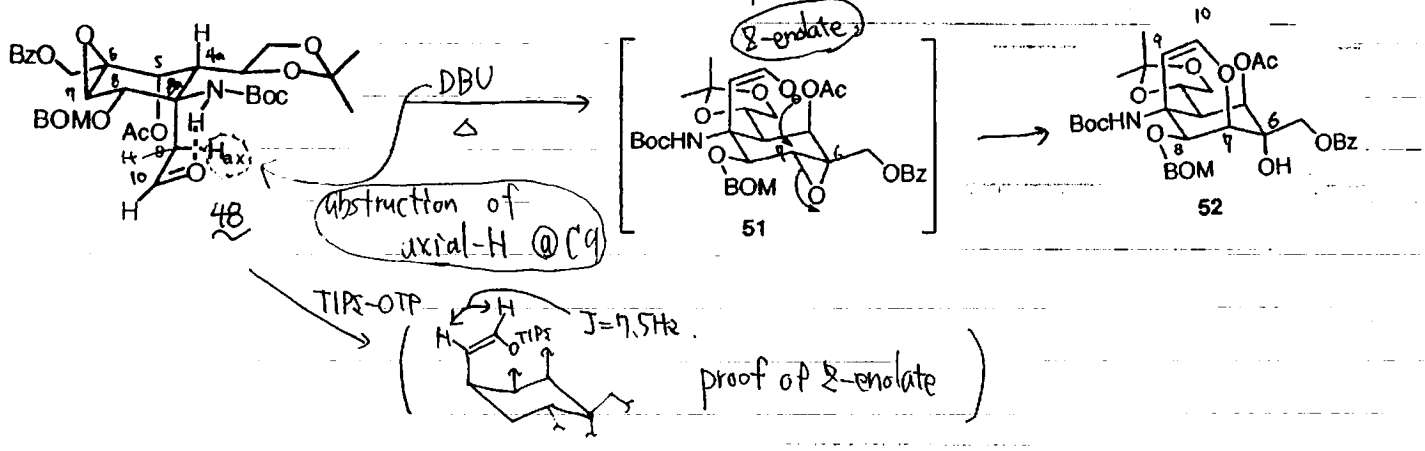
(IBX = 2-Iodoxybenzoic acid )

selectivity 

48 → 49 oxidization of C10 aldehyde to carboxylic acid



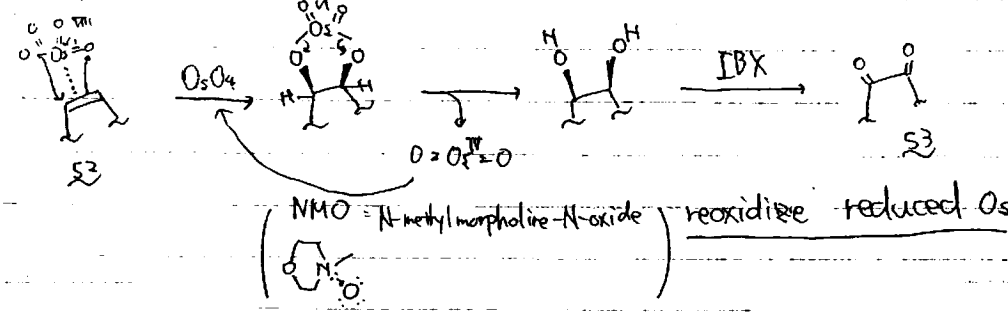
48 → 52 Selective formation of 2 enol and epoxide opening



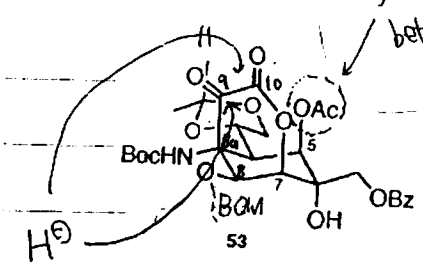
52 → 53 1) formation of diol

2) oxidation of diol to α-diketone

(IBX (ref) 47 → 48 2))

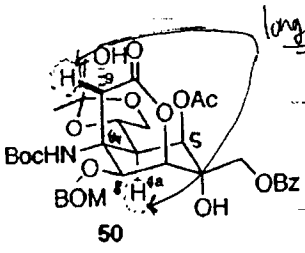


53 → 54



severe steric hindrance between axial -OAc.

$NaBH_4$ / MeOH — quant.



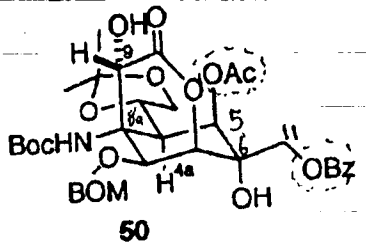
long range coupling → C9-configuration determined

as single product

C skelton ··· fully functionalized correct stereogenic

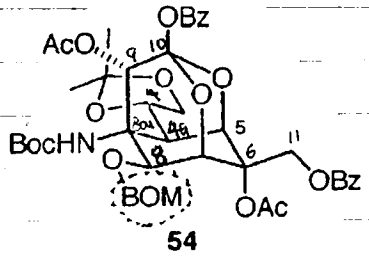
V. Introduction of Guanidine

[Schere 11]



1) Et_3N excess. MeOH (15°C @ 1.2days)

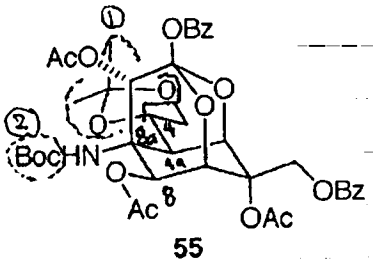
2) $BzCl$ (3.5eq), Et_3N (5eq), Py (5eq) (0°C @ 30min) then Ac_2O excess, DMAP (1eq) (r.t. @ 2hr)



1) H_2, $Pd(OH)_2$-C, MeOH (r.t. @ 12hr)

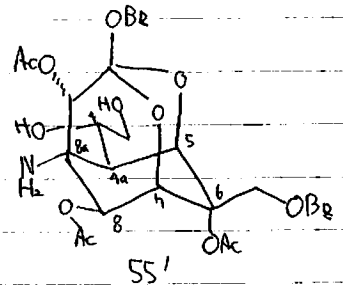
2) Ac_2O, DIMAP (1eq), Py (r.t. @ 2hr)

96% in 2steps

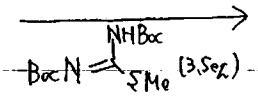


1) TFA (excess) MeOH-H_2O (10/1) (r.t. @ 1.1 days)

2) CAN (1eq) MeCN-H_2O (4/1) (85°C @ 2hr)

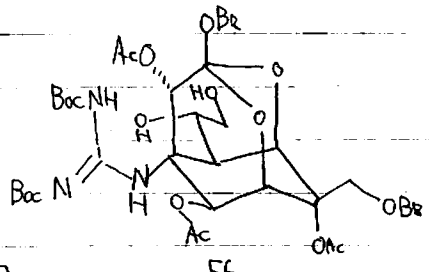


3) $HgCl_2$ (3.5eq) Et_3N-DMF



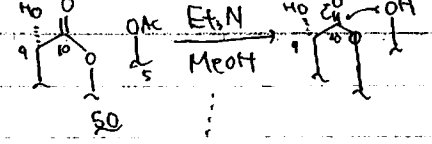
(3.5eq) (r.t. @ 2hr)

55 → 56 53%

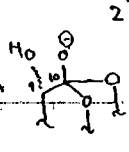


50 → 54 ortho ester forming

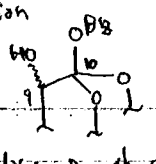
1) Deacetylation @C5 + debenzoylation @C11



temp. ≤ 15°C. prevent from epimerisation @C9

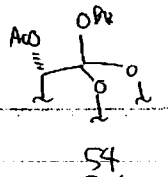


2) Benzoylation (basic) @C10 + @C11



due to acidity of ortho ester C10-OH this site protected preferably (?)

then Acetylation @C9, C6



54 ⟶ 55

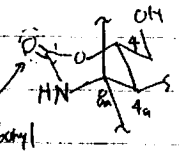
transform C8-OBOM to -OAc

[diminish steric congestion around C8 amino group]

1) Hydrogenolysis (BOM deprotection)

2) Acetylation

55 ⟶ 55' ⟶ 56

1) hydrolysis of the acetonide } conventional acidic method ⟶ 

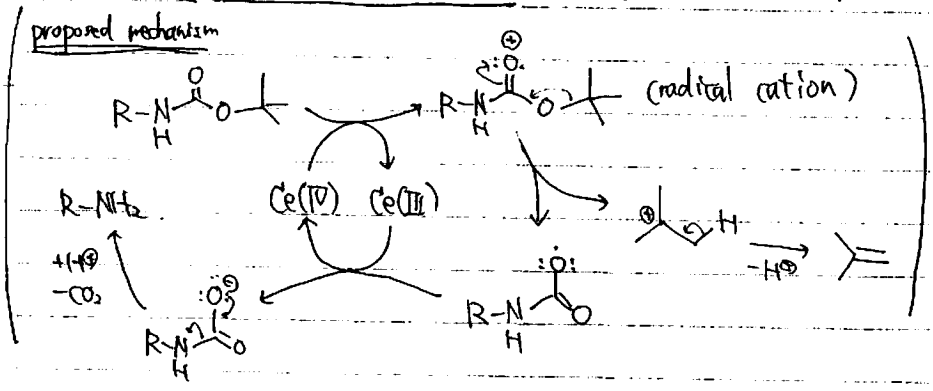
6-membered carbamate.

2) removal of Boc group } two step deprotection necessary

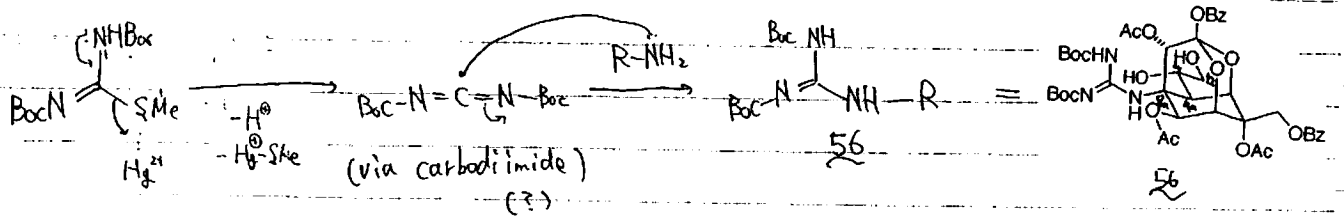
CAN = Ceric Ammonium Nitrate $(Ce^{IV}(NH_4)_2(NO_3)_6$ — neutral condition

role: a one-electron transfer catalist (T.L. 1996, 37, 2035.)

proposed mechanism

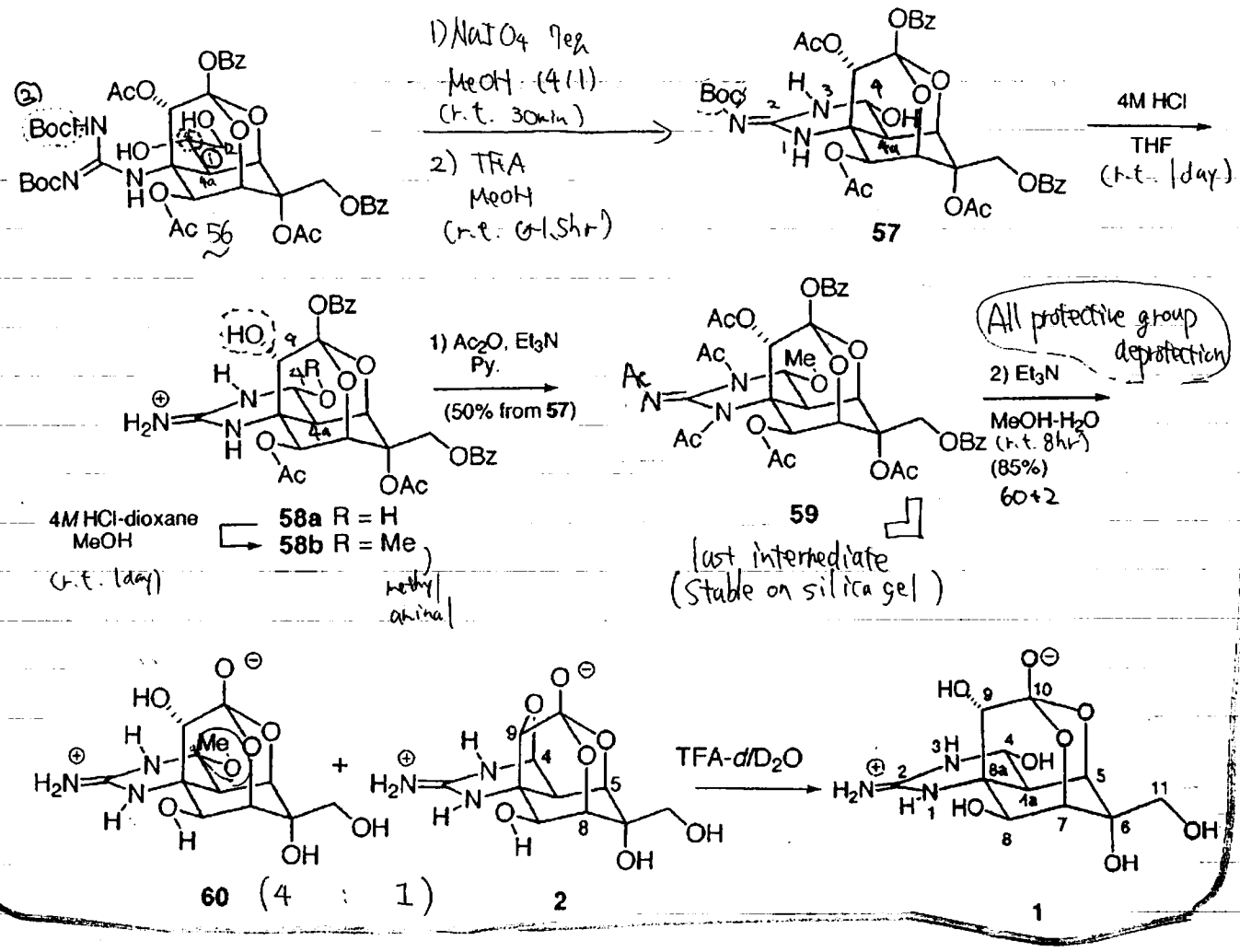


3) $HgCl_2$ ··· complex formation with sulfur atom.



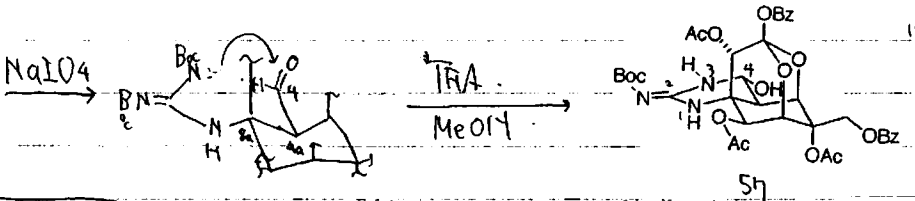
VI Completion of the Total Synthesis

(Scheme 12)



56 ⇒ 57

1) Cleavage of 1-2 diol @C4-C12 2) cyclic guanidine formation & Boc deprotection @N3



^{1}H-NMR different time to time

57 + 57-H mixture

57

57 → 58a

deprotection of remaining Boc group

58a → 58b

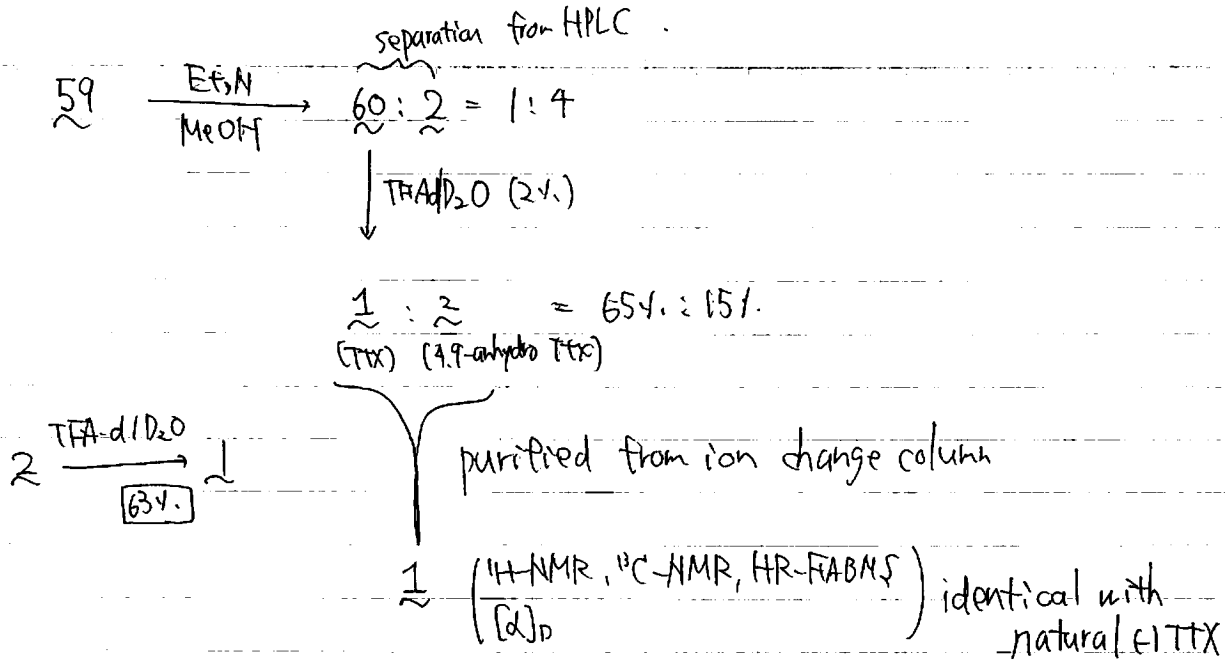
hemiaminal ⟶ hemi-aminal @C4 (stability of aminal ?)

unstale under basic condition

∴ Acyl deprotection of 58a — very difficult (eg NH3-MeOH, 58a is decomposed)

58a → 59

Acetylation @C9 .... Isolation as peracetate


separation from HPLC.
59
Et3N
MeOH
60 : 2 = 1 : 4
TFA·d1D2O (2V.)
1 : 2 = 65V. : 15V.
(TTX) (4,9-anhydro TTX)
2
TFA-d1D2O
63V.
1
purified from ion change column
1
(1H-NMR, 13C-NMR, HR-FABMS
[α]D
identical with natural (-)TTX

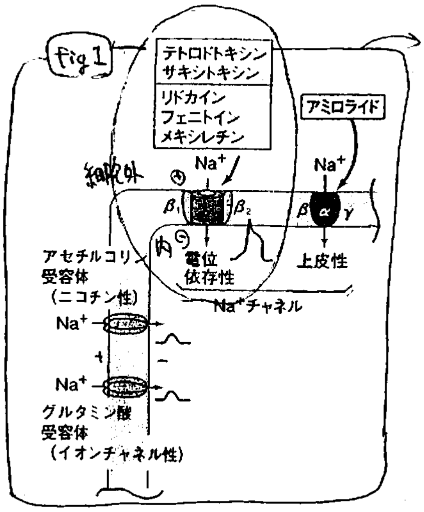
(append)

Biological aspects

《binding features》

TTX … specific binding to voltage dependent Na channel

Fig 1

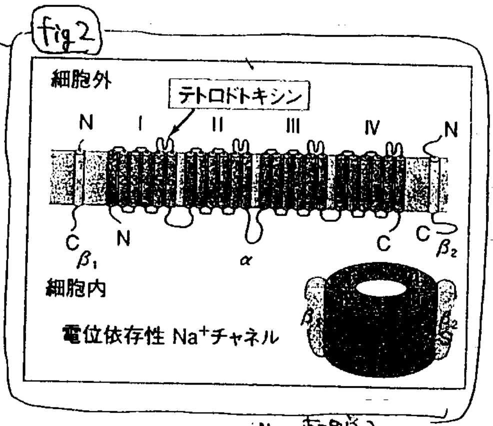


role : open the influx gate
react to action potential

α, $β_1$, $β_2$ three subunit
↑
6回膜貫通単位 (Segment 1~6)
×
4 repeat (I~IV)

TTX binding to a joint between Segment 5 and 6 at Repeat I

fig 2



(New 薬理学)

Q detailed bound structure of TTX to Na channel has not been solved

---

《origin of TTX》

▶ content of TTX differ from { place to place / body to body } → biosynthesized in puffer fish ??

▶ TTX was isolated from many other animals (newt (イモリ), frog, octopus and crab)

* Cultured puffer fish were not toxic ! Matsui (1984)
* TTX was detected in marine animals with a diet of pupper fish Yasumoto (1986)
* TTX-producing bacteria were isolated Yasumoto, Noguchi, Shimizu, et al (1986)
  (vibrio sp. / Pseudomonas sp. etc..)

TTX is
① accumulated through the food chain
② synthesized by intestinal bacteria (腸内) (in body)

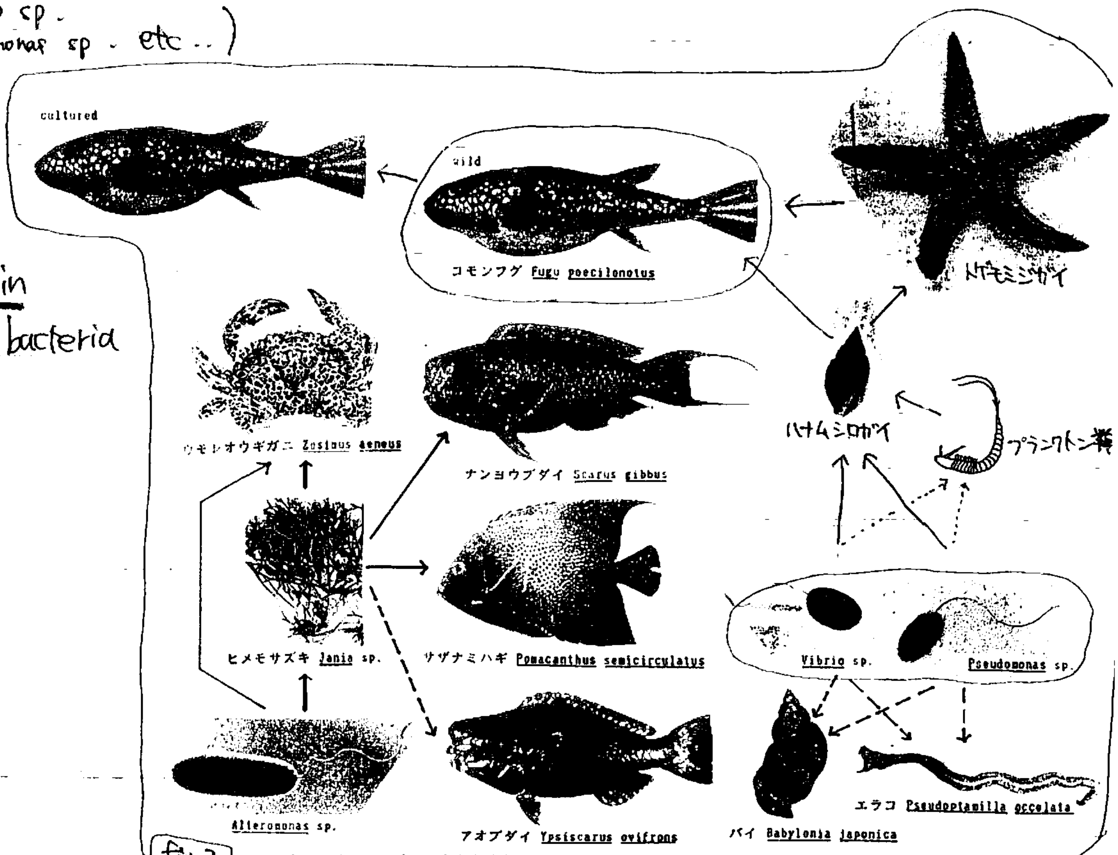


fig 3 テトロドトキシン(TTX)含有生物と毒の移行経路（実線は確認された経路、破線は推定経路）

＜ role of TTX for puffer fish ＞

① TTX is clasified as "toxin" not "venom"
　a weapon for eremy(?) — only a few examples

② a substance for protection　like "skunk's gas"　Matsumura.K.

③ male attaching pheromore at the time of spawning (Nature 1995, 378, 563.)
　産卵

Why isn't puffer fish poisoned with TTX ?

(binding ability $\leq \sim 10^{-3}$)

① difference of Sodium Channel protein between the mammals and them

② TTX-binding protein
　↓ isolated from the blood plasma (Matsui. T. et al. Toxicon 2000, 38, 463.)
　making TTX nonpoisonous ??

③ an enzyme ?
　a special biochemical route ?

表2・1　日本産フグの毒力表（谷，1945）

科名	種類	卵巣	精巣	肝臓	皮	腸	肉	血液
フグ	クサフグ	●	○	●	◎	●	○	
	コモンフグ	●	◎	●	◎	◎	○	
	ヒガンフグ	●	○	●	◎	◎	×	×
	ショウサイフグ	●	×	●	◎	◎	○	
	マフグ	●	×	●	◎	◎	×	
	ノフグ	●	×	◎	◎	◎	×	
	アカメフグ	◎	×	◎	◎	○	×	×
	トラフグ*	◎	×	◎	×	○	×	×
	シマフグ	◎	×	◎	×	○	×	
	ゴマフグ	◎	×	◎	○	×	×	
	カナフグ	×	×	◎	×	×	×	
	サバフグ	×	×	×	×	×	×	
	カワフグ	×	×	×	×	×	×	
	キタマクラ	×		○	◎	○	×	
ハリセンボン	ハリセンボン	×		×	×	×	×	
	イシガキフグ	×		×	×	×	×	
ハコフグ	ハコフグ	×	×	×	×	×	×	
	ウミスズメ	×	×	×	×	×	×	
	イトマキフグ	×	×	×	×	×	×	

すべて最強の毒力を示す．　●：猛毒，10 g 以下で致死的，　◎：強毒，10 g 以下では致死的でない，　○：弱毒，100 g 以下では致死的でない，　×：無毒，1000 g 以下では致死的でない．
*カラスを含む

Anyway ...

Be careful !!
— if you eat puffer fish.

reference) Ann. NY. Acad. Sci. vol. 479
海洋動物の毒　成山堂書店
フグ毒の毒性機構に関する研究　科研費報告書 (2000. 3)